Final Report to the Department of the Environment, Water, Heritage and the Arts

Connectivity of black cod *Epinephelus daemelii* between Elizabeth and Middleton Reefs (as measured by population genetic structure based on microsatellites)

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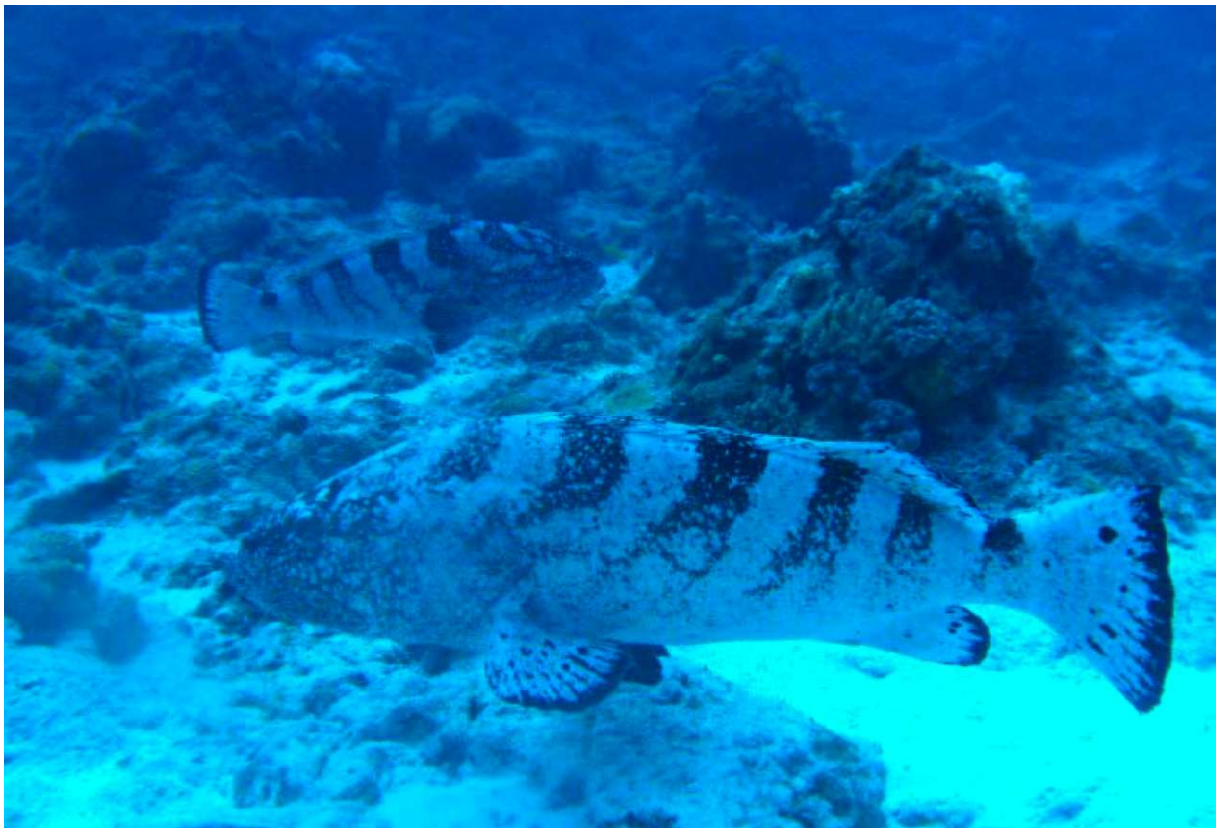

Executive Summary

This study investigated the population genetic structure of black cod (*Epinephelus daemelii*) from Elizabeth and Middleton Reefs in the Elizabeth and Middleton Reefs Marine National Nature Reserve. Six independent microsatellite markers were used to screen individuals from these two reefs for population genetic variation in order to determine if there was any evidence of population genetic structure, which would indicate that Elizabeth and Middleton Reef black cod populations are discrete. An additional original aim was to determine if these reefs were genetically distinct from coastal black cod populations on the Australian east coast, but this could not be achieved since tissue samples of only four coastal black cod individuals were available from Museum specimens.

Six of 17 microsatellite markers tested were screened from 78 Elizabeth and Middleton Reefs individuals and four coastal individuals, totalling 82 black cod. These were all variable with observed heterozygosities (H_o) ranging from 0.45 to 0.95. Not unexpectedly, evidence from the genetic structure suggests that the Elizabeth and Middleton Reefs populations are not distinct and are in fact a single stock. Furthermore, there is a suggestion that Elizabeth and Middleton Reefs populations are not distinct from the coastal population either, although the coastal sample was too small to consider this evidence definitive. This leads to four recommendations from the present study:

Recommendation 1: Additional individuals, especially from the Australian coastal population and additional Norfolk Island and New Zealand sites would be required to make a more comprehensive assessment of the linkages between populations of black cod in the southwest Pacific, so that potential sources of stock replenishment can be identified.

Recommendation 2: Additional microsatellite markers are needed to have greater confidence in our ability to determine if connectivity among these two sites is restricted. The present analysis does not have the statistical power to achieve significant results, due to small sample sizes (fewer than 50 individuals per site), restricted sampling (only two sites) and the use of only six microsatellites in this study. The data from an additional three microsatellites used to screen for the same populations (Appleyard and Ward 2007), when combined with the present data and re-analysed, will achieve greater statistical power in the face of the small sample sizes. This will provide greater confidence in the combined results. Importantly, accepting the null hypothesis (populations are not partitioned among study sites) does not prove that the alternative hypothesis (populations are partitioned among the study sites) is not true, particularly given the small sample sizes and number of markers used in this and the previous study.

Recommendation 3: In the absence of relevant data on the impact of recreational fishing on apex predators such as black cod at Elizabeth and Middleton Reefs, it would be wise to manage these reefs to ensure the maintenance of high standing stocks of these apex predators at Elizabeth and Middleton Reefs. In other words, these reefs should be fully protected no take areas, on the basis of research on apex predator standing stocks in Hawaii (Friedlander and DeMartini 2002), since Elizabeth and Middleton Reefs contains the largest reported Australian standing stock of black cod, an apex predator of the same genus studied in Hawaii.

Recommendation 4: Initiate a study to evaluate the impact of recreational and charter fishing activities in partially fished and no-take reserves at Elizabeth and Middleton Reefs and Lord Howe Island Marine Park. Partially protected areas open to recreational fishing in both Hawaii and the Great Barrier Reef have suffered substantial declines in shark and cod numbers

compared to areas with complete protection (Friedlander and DeMartini 2002, Robbins et al 2006). Fishing at Elizabeth and Middleton Reefs could deplete the abundances and sizes of apex predators such as black cod (and Galapagos sharks) even if they are not target species (as per van Herwerden et al 2008).

Background

Black cod *Epinephelus daemelii*, like all large serranids, has a restricted range and distribution (Heemstra and Randall 1993) and is locally rare (Choat et al 2006). A large scale survey of two offshore reefs, conducted in February 2006 in the Elizabeth and Middleton Reefs Marine National Nature Reserve (EMRMNNR) reported the abundance of *E. daemelii* to be on average three adult individuals per hectare for Middleton Reef and five adult individuals per hectare for Elizabeth Reef (Choat et al 2006). The adults prefer sheltered, lagoonal habitats and are easily visible, making them a potentially easy target for fishing.

This species is protected in the Elizabeth and Middleton Reefs Marine National Nature Reserve and New South Wales state waters due to its perceived rarity (*Fisheries Management Act 1994)* and restricted geographic range. This study aims to examine the connectivity among *E. daemelii* populations from coastal and offshore locations at both Elizabeth and Middleton Reefs so that appropriate management policies may be implemented to ensure the persistence of healthy stocks of this species.

A suite of six microsatellite markers were used to determine if the populations at the reefs are connected by high levels of dispersal (gene flow), which maintains genetic homogeneity, or if they are genetically differentiated, suggesting partitioning of the stocks due to restricted dispersal between locations. Based on levels of polymorphism at the microsatellite markers, an assessment of the retention of genetic diversity at the two reefs was also made. This is an important issue for management of Australian *E. daemelii* resources, since two partitioned populations will each be more susceptible to overfishing than that of a single population. However, even a single population of this species, which is now rare along the east coast of Australia, is likely to be susceptible to overfishing if not protected.

Materials and Methods

Sample collection:
A total of 58 *E. daemelii* individuals were collected from Middelton (42 individuals) and Elizabeth Reefs (16 individuals) in February 2006 during a survey trip undertaken by the Department of the Environment, Water, Heritage and the Arts (see Report by J.H. Choat et al 2006). Since the number of samples collected from Elizabeth Reef in February 2006 was limited, this site was resurveyed and sampled in February 2007 and an additional 20 tissue samples were obtained by CSIRO, bringing the entire sample size to 78, comprising of 31 from Elizabeth Reef and 47 from Middleton Reef. Tissue and/or scale samples were collected *in situ* using a modified biopsy probe developed for sharks (Robbins 2006). These small amounts of muscle/skin tissue (dry weight 0.16mg-79mg) and/or scales were immediately preserved in 80% Ethanol. An additional four *E. daemelii* tissue samples were kindly provided by The Australian Museum, Sydney. These specimens were collected from the NSW and southern Queensland coasts. The coastal samples could not be formally included in analyses, due to the small sample size, but were screened for their genetic profiles and are compared to the EMR populations (Figure 1).

Laboratory procedures:
Total DNA was extracted from tissues or scales using proteinase K digestion and standard salt-chloroform extraction procedures (Sambrook et al. 1989). Overall, 17 microsatellite (Msat) loci were tested (Table 1). These had previously been developed for other serranids (Nugroho et al. 1998; van Herwerden et al. 2000; Zatcoff et al. 2004; Zhu et al. 2005). Initial testing of all loci involved optimisation of PCR (Polymerase chain reaction) amplification for each locus, as different loci require different reaction conditions, particularly the amount of $MgCl_2$ and the annealing temperature (T_a °C). Each 20ul PCR reaction volume contained 2.5mM Tris-Cl (pH 8.7), 5mM KCl, 5mM $(NH_4)_2SO_4$, 200µM each dNTP, 1.5-4.5mM $MgCl_2$, 10µM each primer, 1 unit of Taq Polymerase (Qiagen) and 10ng template DNA. The T_a for each primer pair is shown in Table 1 and is a function of the specific nucleotide composition of the unique primers that define each locus. The amplification involved an initial denaturation step for 2 min at 94°C, followed by 35 cycles at 94°C for 30s, 30s at T_a °C (see Table 1 for details) and 1 min 20s at 72°C. A final extension was done at 72°C for 10 min.

PCR products were checked visually on 1.5% agarose gels. In total nine microsatellite loci were found to produce satisfactory PCR products (Table 1, denoted with *). PCR products for 4-5 individuals per location were purified by isopropanol precipitation and quantified using a spectrophotometer (NanoDrop ND1000 Version 3.3, Nanodrop Technologies Inc. USA) and sent to Macrogen Inc., South Korea for direct sequencing in both forward and reverse directions using the PCR primers. Sequences of all individuals for each locus were aligned using Sequencher 4.5 (Gencode Corporation) and visually checked for polymorphism, both in the flanking and the repeat regions. Initial screening of the nine primer pairs revealed the microsatellite repeat motifs shown (Table 2).

The initial screening of sequences obtained from 4-5 individuals from each location revealed six of the nine loci to be polymorphic due to differences in the number of dinucleotide repeats in *E. daemelii*. There were no differences in the sequences flanking the repeat motifs for any of the nine loci. Therefore all six of the polymorphic loci need to be genotyped in all individuals. The forward primers of these six loci were therefore fluorochrome labelled. PCR reactions were carried out on all 62 individuals (4 Museum and 58 samples collected in February 2006) using the same conditions described above in the presence of 1.5mM $MgCl_2$ and at the specified T_a (as per Table 1). The additional 20 samples, which were later obtained from CSIRO were subsequently screened as per the previous protocols.

A sub-set of samples for all loci was quantified on 1.5% agarose gel using a 25ng PCR standard. This determined the amount of PCR product to be screened by Polyacrylamide gel electrophoresis (PAGE) for genotyping. The six loci were split into two groups of three so that the Forward primer of each locus set could be labelled with either Hex, Tet or Fam fluorochromes. This way they could be pooled for concurrent genotyping per individual sample as follows: (set 1) PM12, Gag45 and Mbo66 and (set 2) Gag23, 2.22 and PM02. The pooled PCR products of both sets of loci were subsequently cleaned to remove unincorporated primers and nucleotides using Sephadex G-50 prior to genotyping (using MegaBACE equipment) at the Genetic Analysis Facility, James Cook University.

Table 1: Seventeen serranid microsatellite loci, defined by specific primer pairs, which were optimized for two individuals from each EMR location. T_a in °C is given for each primer pair. *Denotes the nine primer pairs that produced satisfactory PCR products indicating that they were appropriate to select for a secondary screen to determine if they are suitably polymorphic.

	Primer pairs	Primer sequence 5'>3"	Annealing T_a°C (No. of cycles)	Reference
1.	2.22F *	ggtcctcgtggatgtgtaacc	Ta=56 (35)	(van Herwerden et al. 2000)
	2.22R *	cacatgggatgaacttcagc		
2.	7.90F	atcctaatccacacagaatgc	Ta=53-51	
	7.90R	ggtttaatcgtgtttatgaccg	(5-30)	
3.	9.24F	cacacagcagcaattcttagc	Ta=57 (35)	
	9.24R	ctgatagatatttttgtcc		
4.	10.38F	ctttgggcaatatgtcaa	Ta=50 (35)	
	10.38R	gattatagcatgaattaaacc		
5.	12.46F *	cttgacaactagttccactgg	Ta=59-57	
	12.46R *	gctcgtcttggaaagtcttgg	(5-30)	
6.	PM01F	ctcgctgctttggaggcagtata	Ta=65-63	(Zhu et al. 2005)
	PM01R	tggctcagtaggtggttaaattttagg	(5-30)	
7.	PM02F *	gatcagcctgttagccctggataa	Ta=67-61-59	
	PM02R *	ccccctggccaagtcacag	(3-7-25)	
8.	PM09F	agccgagcgatagatggggag	Ta=65-63	
	PM09R	cgcttgcaacggcgtgttt	(5-30)	
9.	PM10F *	gcggacaggctggaaaactg	Ta=61-59	
	PM10R *	ccgctgactgtgatctcccaa	(5-30)	
10.	PM12F *	agaaaaagctccacaacacaacaa	Ta=61-59	
	PM12R *	gagccccagtcccaaatattg	(5-30)	
11.	EM-01F	tatctggcagaggttttatt	Ta=49 (35)	(Nugroho et al. 1998)
	EM-01R	ttggttcttattgttacttt		
12.	EM-03F	aatacggacacacgcaca	Ta=51-49	
	EM-03R	gaacacgacccctgctaa	(5-30)	
13.	EM-08F *	ccccctctatctctccac	Ta=53-51	
	EM-08R *	caaataaggcacgctctc	(5-30)	
14.	Gag23F *	gcatttgtgttaggatgacact	Ta=55-53	(Zatcoff et al. 2004)
	Gag23R *	cacatggacaggattgagga	(5-30)	
15.	Gag45F *	cctcacgacgagtccaggag	Ta=61-59	
	Gag45R *	gtttgccttaacggatgtctttct	(5-30)	
16.	Mbo48F	caacgttgtcataatctgagcat	Ta=61-59	
	Mbo48R	cgtggatgatgttaactttggtg	(5-30)	
17.	Mbo66F *	cgcatgtttgtaagaacaggaag	Ta=59-57	
	Mbo66R *	gcttcactcttgggttgttgg	(5-30)	

Table 2: Repeat motifs for nine primer pairs from direct sequencing, indicating heterozygosity (he) or homozygosity (ho) of these microsatellites due to changes in repeat number (as shown) and the fragment sizes obtained from *E. daemelii* individuals. Based on heterozygosity six loci (identified by ^) were selected to be fluorochrome labelled and used to screen the *E. daemelii* populations for connectivity.

Loci	Repeat Motifs	Initial No. of alleles	Size (bp)
2.22^	$(CA)_{28}$ and $(CA)_{26}$	2 (he)	140-200
12.46	$(TG)_6$	1 (ho)	350
PM02^	$(CA)_8$; $(CA)_9$; $(CA)_{10}$ and $(CA)_{12}$	4 (he)	170-200
PM10	$(CA)_7$	1 (ho)	170
PM12^	$(TG)_{17}$; $(TG)_{22}$; $(TG)_{24}$ and $(TG)_{27}$	4 (he)	160-180
Gag45^	$(GT)_{14}$; $(GT)_{15}$;$(GT)_{16}$; and $(GT)_{17}$	4 (he)	150-200
Gag23^	$(GT)_{12}$ and $(GT)_{13}$	2 (he)	90-120
Mbo66^	$(GT)_{11}TA(GT)_4$; $(GT)_{12}TA(GT)_2$; $(GT)_{13}TA(GT)_3$ and $(GT)_{15}TA(GT)_3$	4 (he)	110-150
EM-08	$(AC)_7AAA(AC)_4$	1 (ho)	250

Genotype scoring and Analysis:

Individual genotypes for all six loci were scored using FragmentProfiler Version 1.2 (MegaBACE, Amersham Bioscience 2003). Number, frequency and size of alleles were recorded for each locus. We could not include the small number of coastal samples (4) in further analyses, due to the severe sample size imbalance. However, we do indicate what allele frequency was represented in these few individuals (Figure 1). The data from the two study populations from Elizabeth and Middleton Reefs were further analysed, in two stages, initially without the additional 20 sample collection obtained from CSIRO and subsequently with the additional CSIRO obtained samples. Henceforth, these will be referred to as *Analysis 1* and *Analysis 2* respectively. All loci were tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GenePop web version 3.4 (http://genepop.curtin.edu.au/) (Raymond and Rousset 1995). This is important, as loci that are not in HWE and loci that are linked (evidenced by LD) have to be excluded from further analysis in order to conform to the assumptions underlying population genetic analyses. Therefore, loci that display significant excess or deficiency in observed heterozygosity (Ho) relative to the expected heterozygosity (He) can not be used in population genetic analyses, as they do not conform to HWE expectation. Observed and expected heterozygosities were determined in Arlequin Ver. 3.1 (Excoffier et al. 2005), so that loci that do not conform can be excluded from further analysis.

Allele frequencies and F_{is} values (measure of within population heterozygote deficit due to inbreeding) of each locus that conformed to assumptions were calculated for each location using FSTAT Ver. 2.932 (Goudet 1995). Additionally, allele frequencies were plotted for each locus and location so that differences in allele frequencies between populations may be apparent. If differences between locations are observed this suggests that there may be restricted connectivity (low gene flow) among sites. The fixation index, F_{st} measures *E.daemelii* genetic differentiation among locations and was obtained by AMOVA analyses implemented in Arlequin Ver. 3.1 (Excoffier et al. 2005).

Results

Of the nine loci that successfully amplified, only six loci were polymorphic. Therefore only these six loci were used to investigate genetic connectivity among Elizabeth and Middleton Reef populations of *E. daemelii*. Of the six loci, two had large numbers of alleles, PM-12 with 22 and 2.22 with 16 alleles. The remaining four loci had five or fewer alleles, Mbo66 had five, Gag45 and Gag23 each had four, whilst PM-02 only had three alleles.

Hardy-Weinberg exact tests in *Analysis 1* revealed that all six loci were in equilibrium ($p>0.05$) for both populations, as also shown by Ho and He values for each locus (Table 3A), none of which were significantly different for any of the six loci. Neither were any of the six loci in LD. Therefore all six loci were retained for population genetic analyses. However, in *Analysis 2*, three loci failed to generate scorable data (PM12, Gag45 and 2-22) in the new samples and two of the 20 samples were excluded due to insufficient data for the remaining three loci (PM2, Mbo66 and Gag23). As a result, these three loci alone could be analysed for the additional 18 specimens. There was evidence that the Elizabeth Reef population, now containing 31 individuals and three of the six loci, was not in Hardy Weinberg equilibrium for one of the three loci (Mbo66, Table 3B). Consequently, all further analyses, results and discussion pertain to *Analysis 1* only (6 loci for 42 Middleton and 16 Elizabeth Reef samples), not including the additional 2007 samples. The alternative option of analysing all samples (47 and 31 for Middleton and Elizabeth Reefs respectively) for only two loci (or one third of the data) would be less likely to yield results that could be confidently used to infer population genetic structure.

In *Analysis 1* the overall F_{is} of all six loci combined was -0.084 for Middleton Reef and -0.149 for Elizabeth Reef. Low estimates of F_{is} indicate that mating is essentially random within the cod populations and that there is no localised assortative mating (mating of individuals having more traits in common than likely in random mating). The global estimate of F_{st}, which measures genetic partitioning (or the level of gene flow) from combined data for all six loci, was -0.00796 with a *P*-value of 0.823, suggesting that there is no partitioning of genetic variance between Elizabeth and Middleton Reefs. This shows that these cod populations are likely to exhibit high levels of gene flow. The locus by locus AMOVA also revealed lack of partitioning and thus high levels of gene flow between populations, ranging between F_{st} of -0.00218 (2.22) to -0.02073 (Gag23).

Plotting allele frequencies of all loci for each population (Figure 1) illustrates these results visually, showing that locus specific alleles were distributed equally amongst both locations. The four coastal samples were also plotted here, in order to determine if there are any obvious differences in the alleles present in coastal populations compared to the EMR population. From this comparison we tentatively conclude that the coastal samples appear to be genetically very similar to the EMR population. However, as sample size is minimal for the coastal population, this should be considered very preliminary evidence for connectivity between coastal and EMR populations of *E. daemelii*.

Table 3: Genetic diversity for six *E. daemelii* loci screened from Elizabeth and Middleton Reefs. Number of individuals sampled (n) per reef, number of alleles (k) per locus, expected (He) and observed heterozygosity (Ho), F_{is} (correlation of alleles within individuals). A) Analysis 1 and B) Analysis 2, asterisk indicates that Hardy-Weinberg equilibrium was not observed ($p<0.00001$) for this locus in this population, nd indicates that this calculation was not performed, as two remaining loci in this dataset were insufficient to warrant further investigation of population genetic differentiation between Elizabeth and Middleton Reefs when including the additional Elizabeth Reef sample.

A)

Locus	Middleton Reef	Elizabeth reef
PM-12		
n	42	16
k	19	13
He	0.93345	0.91734
Ho	0.95238	0.9375
F_{is}	-0.021	-0.069
Gag45		
n	42	16
k	4	3
He	0.47217	0.45766
Ho	0.5	0.5625
F_{is}	-0.06	-0.239
Mbo66		
n	42	16
k	5	3
He	0.61245	0.56653
Ho	0.71429	0.8125
F_{is}	-0.169	-0.455
Gag23		
n	42	16
k	4	4
He	0.51641	0.52621
Ho	0.53659	0.5625
F_{is}	-0.04	-0.071
2.22		
n	42	16
k	15	14
He	0.89013	0.90726
Ho	1.0	1.0
F_{is}	-0.125	-0.106
PM-02		
n	42	16
k	3	3
He	0.41193	0.48589
Ho	0.45238	0.5
F_{is}	-0.1	-0.03

B)

Locus	Middleton Reef	Elizabeth reef
PM-12		
n	47	
k	19	Excluded due
He	0.93345	to insufficient
Ho	0.95238	data
F_{is}	-0.021	
Gag45		
n	47	
k	4	Excluded due
He	0.47217	to insufficient
Ho	0.5	data
F_{is}	-0.06	
Mbo66		
n	47	31
k	5	3
He	0.61245	0.60911
Ho	0.71429	0.88571*
F_{is}	-0.169	nd
Gag23		
n	47	31
k	4	4
He	0.51641	0.51252
Ho	0.53659	0.61111
F_{is}	-0.04	nd
2.22		
n	47	
k	15	Excluded due
He	0.89013	to insufficient
Ho	1.0	data
F_{is}	-0.125	
PM-02		
n	47	31
k	3	3
He	0.41193	0.44562
Ho	0.45238	0.50000
F_{is}	-0.1	nd

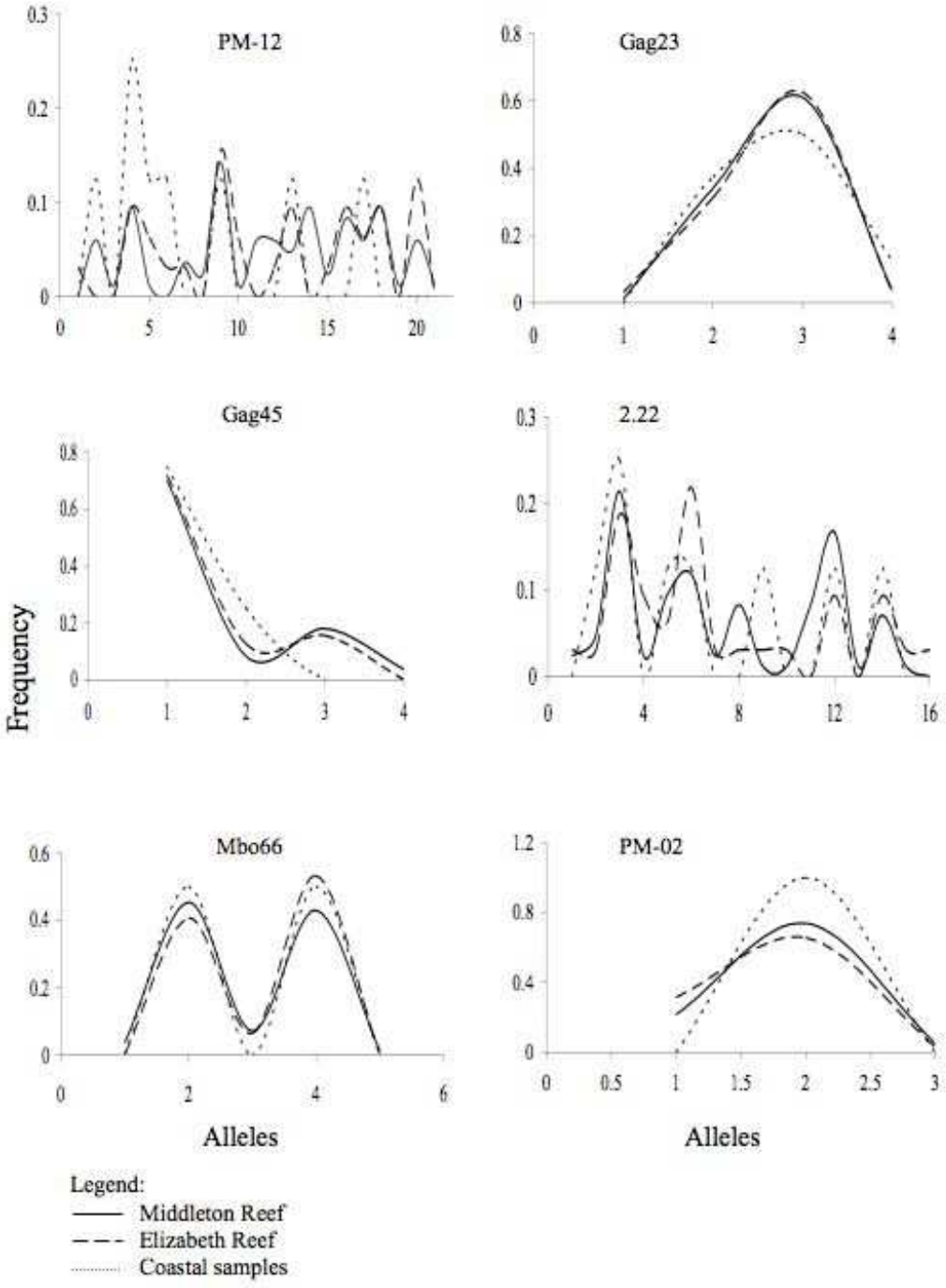

Figure 1: *Analysis 1* Allele frequency distributions *of E. daemelii* from Elizabeth and Middleton Reefs for each of the six microsatellite loci screened. Note that allele frequencies of coastal samples from Queensland/NSW are also shown for comparative purposes.

Discussion

This study is part of a larger collaborative study that aims to investigate the connectivity between populations of *E. daemelii* from EMR and coastal NSW-southern Queensland. As such, the results and conclusions presented here are interim in nature and should be revisited once additional data from an additional three microsatellite loci (generated by CSIRO) has been combined with the data presented here and analysed as a whole. This will allow for six loci in 47 and 31 Middleton and Elizabeth Reef samples respectively, to be analysed as a comprehensive data set. Alternatively, it will allow for nine loci in 42 and 16 Middleton and Elizabeth Reef samples to be analysed respectively. Either way, there will be greater statistical power to determine population structure based on the observed genetic partitioning than could be achieved in the present study alone.

That said, we conclude from the present study that there is no evidence of population subdivision between Elizabeth and Middleton Reefs. Furthermore, we conclude even more tentatively from this work that there is no population partitioning between EMR and coastal populations from NSW-southern Queensland. However, these results remain tentative and should not be used as a basis for sound management policies at present due to a lack of samples from the NSW-Queensland coast.

Once additional data is obtained from our collaborators, this combined data will be re-analysed and a final report that is more conclusive will be provided, in collaboration with CSIRO. In this light, we make four recommendations pertaining to the management of *E. daemelii* populations at the Elizabeth and Middleton Reefs Marine National Nature Reserve.

Concluding remark and recommendations: It is imperative to note that the healthy populations of black cod at Elizabeth and Middleton Reefs (Choat et al 2006) are likely to suffer reductions if fishing is permitted in these areas, even if it is only recreational and if the black cod are not themselves the target, since black cod at these locations are not accustomed to human activities and are curious towards both divers and baited hooks. Although this is an untested hypothesis at Elizabeth and Middleton Reefs, it has been tested in Hawaii, where there is clear evidence that fishing pressure (including recreational and subsistence fishing) in the main Hawaiian Islands has led to drastic reductions in number and size of numerous fish species, mainly apex predators, including the Hawaiian grouper, *Epinephelus quernus* and sharks. In contrast, these species are still common at the remote North Hawaiian Islands, which are lightly fished (Friedlander and DeMartini 2002). Importantly, Friedlander and DeMartini (2002) found that fully protected no-take reserves in the main Hawaiian Islands had higher standing stocks of apex predators and other reef fishes than areas with partial or no protection from fishing, even if these areas were only fished recreationally.

Recommendation 1: Additional populations from other locations should be incorporated into the study of black cod stock structure for informed management to be most effective. These additional samples (ideally represented by at least 50 individuals per location) should be screened with the same suite of nine microsatellites and cyt b from the mitochondrial DNA, used in the collaborative study (this report and Appleyard & Ward 2007). This will allow the establishment of what the level of connectivity is among black cod from Elizabeth and Middleton Reefs, more distant sites such as coastal NSW-southern Queensland, Norfolk Island and New Zealand. Importantly, such increased sampling will allow us to identify source-sink relationships among black cod populations.

Recommendation 2: More markers (microsatellites) are needed to have greater confidence in our ability to determine if there is restricted connectivity among these two sites. The present analysis does not have the statistical power to achieve significant results. This is in part due to small sample sizes (fewer than 50 individuals per site), restricted sampling (only two geographically close sites) and the use of only six markers in this study. The data from an additional three microsatellite loci screened for the same populations (as per Appleyard and Ward 2007) should be combined with the present data and the whole re-analysed to achieve greater statistical power in the face of the small sample sizes. Greater statistical power will allow greater confidence in the analytical results. Please note that in the absence of sufficient data, the acceptance of the null hypothesis (populations are not partitioned among study sites) does not prove that the alternative hypothesis (populations are partitioned among the study sites) is not true. It is critical for effective sustainable management of these black cod populations to have greater statistical power to resolve this and confirm whether black cod from Elizabeth and Middleton Reefs should be managed as a single stock or not.

Recommendation 3: In the absence of relevant data for the impact of recreational fishing on apex predators at Elizabeth and Middleton Reefs, it would be advised to manage these sites in a manner that will ensure higher standing stocks of these apex predators. In other words, these reefs should be awarded fully protected no take status, on the basis of the Hawaiian research on apex predator standing stocks, since Elizabeth and Middleton Reefs have the largest reported Australian standing stocks of Galapagos sharks and black cod, both apex predators of the same groups that were studied in Hawaii.

Recommendation 4: Initiate a study to evaluate the impact of recreational and charter fishing activities in partially fished and no-take reserves at Elizabeth and Middleton Reefs Marine National Nature Reserve and Lord Howe Island Marine Park. Partially protected areas open to recreational fishing in both Hawaii and the Great Barrier Reef have suffered substantial declines in shark and cod numbers compared to areas with complete protection (Friedlander and DeMartini 2002, Robbins et al 2006). Fishing at Elizabeth Reef could deplete the abundances and sizes of these apex predators, even if they are not target species (as per van Herwerden et al 2008).

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